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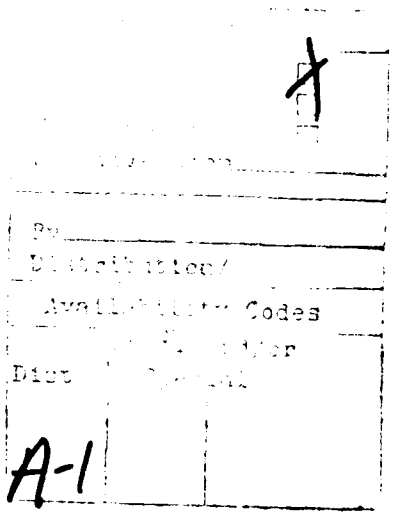

The Tautomeric Equilibria of Thio-Analogues of Nucleic Acid Bases. Part I. 2-Thiouracil: Background, Preparation of Model Compounds, and Gas Phase Proton Affinities

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Summary The preparation is reported of all four of the mono-alkyl derivatives of 2-thiouracil, and of four of the six possible dialkyl derivatives required as models for study of the tautomeric equilibria by physical methods. Gas phase proton affinities are determined using ion cyclotron resonance mass spectrometry, and used to provide quantitative estimates of individual tautomer stabilities in the vapor state. These quantitative results agree well with qualitative deductions of predominant structures for the monoalkyl derivatives from IR spectroscopy.

The concept of heterocyclic tautomerism[1] is critical to the structure of DNA. The correct hydrogen bonding between the base-pairs of the nucleotides, and hence a specific tautomer, is needed for the formation of the double helix.[2,3] For replication to occur, the alpha-helix must unwind to allow for new base-pairing. At this point, should a tautomeric shift of a nucleotide occur the unnatural tautomer could pair with the wrong complementary base causing a mutation[3,4] of the original nucleic acid.

The tautomeric equilibria of uracil (1) are well studied.[1,5,6] 2-Thiouracil (2) has recently been the subject of considerable interest: it inhibits hyperthyroidism in man,[7-9] has been isolated[10] from E. coli. t-RNA, and inhibits virus[11] and bacterial growth,[12] by causing alterations[13] in protein synthesis. Although the effect on protein synthesis is thought[13] to occur by misrecognition of 2-thiouracil as cytosine, the process is not well understood. 2-Thiouracil is also of interest because of mutagenic,[14] anticancer,[15] and antithyroid activity,[16] kidney stone formation inhibition,[17] and antidote properties for mercury poisoning.

(Block 1)

The present paper records the results of work aimed at the quantitative elucidation of the tautomeric equilibria of 2-thiouracil and its mono alkyl derivatives. Detailed studies[18,19] of the tautomerism of uracil (1) and of each of the thiouracils[20] (2-4) have shown that in the solid, in solutions, in low temperature matrices, and in the vapor, the prevalent tautomer is in each case the dioxo, thione-oxo, or dithione form shown (1-4). Recently, studies on substituted 4-thiouracil derivatives[21-23] and 2-thiouracil derivatives[19f,22] have appeared.

By contrast, there have been few quantitative studies of the precise equilibrium relationships between the predominant tautomer and the various minor forms (Scheme 1). Uracil has been studied in this way and a value of K_T (the tautomeric equilibrium constant) of ca 5000 was deduced.[23]

(Scheme 1)

The present work is aimed at the quantitative study of the tautomeric equilibria of 2-thiouracil in the vapor phase and in solution in diverse solvents. 2-Thiouracil can exist in six tautomeric forms with completed cyclic conjugation (aromatic tautomers) as shown in Scheme 1 (non-ring conjugated tautomers also exist, but are likely to be of little importance[1]). To understand such a complex system of equilibria, it is necessary to study simpler systems in which some of the possibilities are blocked. 2-Thiouracil can form four mono O-, S-, or N-methyl derivatives, each of which can exist in three tautomeric forms (Scheme 2). We now report the synthesis of all four mono alkylated models, as well as four out of the six possible types of dialkyl derivatives corresponding to the six tautomers of Scheme 1, together with a study of the proton affinities of all these compounds by ion cyclotron resonance mass spectrometry. A complilmentary publication for the present paper has studied the tautomeric equilibria by infrared spectroscopy.

(Scheme 2)

Preparation of Compounds.- Alkylation of 2-thiouracil (2) (pKa = 7.75[24], cf. uracil pKa = 9.45) under basic conditions is known to lead mainly to S-alkylation. Under Barrett's conditions,[25] but with only a small excess of methyl iodide and NaOH, (2) gave (6) and (11) (Scheme 3). Similarly, reaction with excess methyl sulfate gave mostly (9) and (11) with compound (10) also isolated in a small amount, although (10) was not reported in the original paper.[26] O-Methyl derivatives were also detected in the reaction mixture in small amounts.

(Scheme 3)

O-Alkylated derivatives have also been reported from the direct alkylation of 2-thiouracil. Thus, methyl phosphate in the presence of triethylamine was stated to give[27] O,S-dimethyl-2-thiouracil (13) (6%), although the presumed intermediate (19) was not isolated. Under phase transfer conditions, O,S-diethyl-2-thiouracil (66 %) was obtained[28] while n-propyl bromide in DMSO and K_2CO_3 gave[29] 1-propyl-2-thiouracil. We used the three step procedure (Scheme 3), previously described for O-ethyl-S-methyl-2-thiouracil[30] to prepare the O,S-dimethyl analogue (13). The transformation of (6) into the chloro-derivative (12) according to Matsukawa[31] was accompanied by a rearrangement into a N-derivative, thus necessitating chromatographic purification.

(Scheme 4)

A similar method was envisaged for the preparation of O-methyl-2-thiouracil (19) (Scheme 4): Step (14) --> (16) is described in the literature;[32,33] we observed that the reaction of 2,4-dichloropyrimidine with

sodium methoxide gave also a small amount of the 2-methoxy-4-chloro isomer. Although replacement of chlorine for sulfur in 2-chloro-4-ethoxypyrimidine is reported[33] (albeit in poor yield), we were unable to achieve such a reaction of thiourea with the 4-methoxy derivative (16), as rapid rearrangement of the methyl from oxygen to nitrogen or sulfur was favoured over production of the desired (19). Hilbert and Johnson[34] report that the tendency of a methoxy group to undergo such rearrangement is much higher than that of an ethoxy group. Use of sodium hydrosulfide instead of thiourea for the transformation of (16) gave only 2,4-dithiouracil (21) (Scheme 4). Reaction of the chlorine in (14), with NaSH, leads[24] to (21), but the smooth exchange also of the methoxy group in (16) is surprising. No (19) was observed, implying that even if the reaction goes through O-methyl-2-thiouracil, exchange of the methoxy group is faster than the initial replacement of the chlorine atom. Use of N,N'-dimethylthiourea in place of thiourea, gave product (20) in quite good yield (Scheme 4). Unexpectedly, compound (20) was resistant to hydrolysis and remained unchanged, even after refluxing with KOH solution, although slow total decomposition was observed.

It was thus necessary to prepare a compound analogous to (16) which would not rearrange under the reaction conditions employed. Compound (15) was synthesised by a similar route to that used for compound (16): nucleophilic substitution by the neopentyl alkoxide. Subsequent reaction with N,N'-dimethylthiourea gave the expected compound (17) in low yield with (18) as the main reaction product (Scheme 4). Because of the stability of (20), compound (18) is possibly not an intermediate on the pathway from (15) to (17).

(Scheme 5)

N(1)-methyl (28) and N(3)-methyl-2-thiouracil (29) were obtained by the three step method of Warrener[35] (Scheme 5): the starting dithiocarbamic acid derivatives, (22) and (23), were obtained by the procedure of Maths[36,37] from ammonium dithiocarbamate.

Gas Phase Proton Affinity Measurements. The comparison of proton basicities of potential tautomeric compounds with those of fixed alkyl derivatives of the various potential tautomers is a standard method for the quantitative investigation of solution phase tautomeric equilibria.[38] Several extensions of this method to the semiquantitative investigation of vapor phase equilibria of tautomeric heterocyclic compounds have been made.[39] Gas phase proton affinity determinations can be carried out using two different methods. The first requires a proton transfer equilibrium of a test compound (Y) of known proton affinity/gas phase basicity with a sample compound (X), for which the proton affinity is to be determined:

$$YH^+ + X \underset{k_r}{\overset{k_f}{\rightleftharpoons}} XH^+ + Y \qquad (1)$$

The equilibrium constant and the free enthalpy ΔG of this reaction can be calculated either by determining the forward and the reverse rate constants, k_f and k_r, or by measuring the concentrations of YH^+ and XH^+ after reaching equilibrium, using ion cyclotron resonance spectrometry[39,40] or high pressure mass spectrometry.[41] In this way, the gas phase basicity of the compound X can be determined. The entropy change, ΔS, can be obtained by studying the rate constants over a wide range of temperatures,[41] or from calculations which take into account symmetry changes in the reaction. The proton affinity of the compound X, defined as $-\Delta H$ of the reaction of Eq. (2) can then be calculated from ΔS and the obtained gas basicity, ΔG of this reaction.

$$X + H^+ \text{ ---> } XH^+ \qquad (2)$$

The second method is based on bracketing experiments.[40] Test compounds (Y_1, Y_2, Y_3,...,Y_n) of known proton affinity can be mixed with the sample compound, and the direction of proton transfer can be determined:

$$Y_1H^+ + X \text{ -----> } XH^+ + Y_1 \qquad (3)$$

$$Y_2H^+ + X \text{ -----> } XH^+ + Y_2 \qquad (4)$$

$$Y_3H^+ + X \text{ -----> no proton transfer} \qquad (5)$$

$$Y_3 + XH^+ \text{ -----> } X + Y_3H^+ \qquad (6)$$

In this way the proton affinity of the compound X can be found to lie between a lower and an upper limit:

$$PA(Y_2) < PA(X) < PA(Y_3)$$

The proton affinities of the test compounds used in the following bracketing experiments were obtained from Ref. 40. For example, bracketing experiments showed that protonated 2-thiouracil transferred a proton to aniline, but protonated m-chloroaniline transferred a proton to 2-thiouracil. Therefore the proton affinity of this compound must be between the proton affinities of m-chloroaniline and aniline:

208.6 kcal/mol < PA(2-thiouracil) < 209.5 kcal/mol.

The proton affinities of the other compounds were bracketed in an analogous way, and the results thus found are gathered in Table 1.

Discussion of proton affinities. - For each of the monomethyl derivatives, it can be seen that three of the dimethyl derivatives each provide models for one of the three tautomeric forms for that monalkyl derivative (cf. Scheme 2). Thus, for the S-Me compound **(6)**, these three models are the N1-S (cf. **6a**), the N3-S (cf. **6b**), and the S-O (cf. **6c**). The tautomer with the lowest proton affinity will predominate in the gas phase, just as that of the lowest basicity dominates in solution.[1] Hence, the proton affinity of the monoalkyl derivative is expected to be somewhat higher than that of the dimethyl model with the lowest proton affinity, and the monoalkyl compound should exist predominantly in the form of this model.

However, a simple application of the above reasoning is precluded for two important reasons. Firstly, the substitution in OH, SH, or NH of an alkyl group for the hydrogen to give OR, SR or NR has a significant effect on the vapour phase basicity. Secondly, it is assumed implicitly that each of the model compounds forms a cation of similar structure.

Effects of S-, N-, and O-Methylation on Gas-phase Basicities. Some relevant data are listed in Table 2. The proton affinity of 2-mercaptopyridine (known to exist as such in the gas phase) is raised by 0.4 kcal/mol in 2-methylthiopyridine.

From Table 2, the effects of N-methylation are seen to be in the range of 3-10 kcal/mol: however, a more precise estimate is provided by our own data. Compounds **2a** and **5b** are known to exist predominately in those forms in the gas phase by IR-measurements,[20a] and they form cations of similar structure (vide infra). Hence, the difference in their proton affinities, 5.0 kcal/mol forms a good value to take as the effect of NMe.

Earlier work from one of our groups[42] has indicated that in the 1-methyl-2-pyridines / 2-methoxypyridines systems the effect of OMe is 2.4 kcal/mol more base strengthening than that of NMe. Although this would indicate 7.4 kcal/mol as the effect of O-methylation, based on the data of Table 2 and other literature results, we believe that this is too high and take 5.0 kcal/mol.

Structures of Cations. 2-thiouracil can form four monocations in which the cyclic conjugation is preserved: 30 - 33. Calculations[43] indicate that cation 31 is more stable than 32 and 33 by ca 3 kcal/mol and so that these are more stable than 30 by ca 2 kcal/mol. We have therefore assumed that cations of type 31 will be formed in the gas phase unless this is procluded by 3-methylation, in which case type 32 and 33 will be formed.

(Block 2)

Quantitative Assessment of Stabilities of Individual Tautomers. In Scheme 6 the proton affinities are deduced for of the individual tautomers of 2-thiouracils by using the S-, N-, and O-methylation increments deduced above. The relative proton affinities listed in Table 3 now provide a measure of the relative stabilities of these four tautomers. Table 3 also lists relation ΔH_f values obtained by calculations.[43]

Table 3 and 4)

A similar treatment is carried out in Table 4 for compounds forming cations of types 32 or 33. The two sets of deductions (Tables 4 and 5) are in fair agreement and together indicate that the stability order:

2a >> 2e, 2d >> 2b.

From the infrared spectroscopic results on the monoalkyl derivatives in the gas phase[20a] for following stability orders can be deduced:

	2a	**>> 2b, 2c**	(from 5)
	2d, 2e	**>> 2b**	(from 6)
	2a	**>> 2d, 2f**	(from 7)
	2e	**>> 2c, 2f**	(from 17)
i.e. overall:	**2a**	**>> 2d, 2e >> 2b, 2c, 2f**	

which is in good agreement with our results.

Conclusions. We conclude that the proton affinity measurements allow semiquantitative estimates of individual tautomer stabilities in the gas phase. We consider that the present work is of particular importance in providing points of reference for theoretical calculations, as is done in the following paper.[43]

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EXPERIMENTAL

Mass Spectrometry. Experiments were performed on a Nicolet FT/MS-1000 Fourier transform ion cyclotron resonance mass spectrometer[44,45]. Thiouracil derivatives were inserted into the high vacuum system using a solids probe. The probe was heated until a sufficient sample pressure had been achieved in the system (usually 5 x 10^{-8} torr). Liquid test compounds for bracketing experiments were introduced through a precision leak valve from a gas/liquid inlet after several freeze-pump-thaw cycles. After electron impact (50 eV) ionization, protonation of the thiouracil derivative of interest was achieved by allowing the fragment ions to protonate the parent molecule during a certain reaction time (usually 500ms - 1s). After protonation, the protonated molecule was "selected" by ejecting all other ions out of the reaction region, using swept r.f. ejection pulses. The selected ion was again allowed to react with the test compound, which had about the same partial pressure as the parent compound. Proton transfer from the selected protonated thiouracil derivative (TH^+) to the test compound (Y) was then studied at various reaction times:

$$TH^+ + Y \text{ -----> } YH^+ + T \qquad (6)$$

Proton transfer from a protonated test compound (YH^+) to the thiouracil derivative was studied by protonating the test compound first, and then allowing it to react with the thiouracil neutral after the selection process described above:

$$YH^+ + T \text{ -----> } TH^+ + Y \qquad (7)$$

The ion selection process [42] was quite useful in eliminating unwanted protonated species (fragment ions, etc.), whose reactions would complicate study of proton transfer from the desired species (YH^+ or TH^+) during the second reaction period. For all compounds studied, only the proton transfer reactions of interest (reactions (6) and/or (7)) were observed after ion selection.

Two thiouracil derivatives of interest possessed proton affinities very close to that of a test compound, and thus both forward and reverse proton transfer reactions (6) and (7) were observed. In these cases the proton affinity assignment was made by studying the relative intensity of each protonated species (YH^+ and TH^+) after a fixed reaction time, to determine which was favored as the proton transfer came to equilibrium.

^1H N.m.r. and ^{13}C n.m.r. were obtained on Varian EM 360 L (60 MHz) and Jeol FX 100 (100 MHz) spectrometers, respectively; unless otherwise noted, chemical shifts (delta) are recorded downfield of tetramethylsilane as an internal standard. I.r. spectra were recorded on a Perkin Elmer 283 spectrometer. Melting points were measured on a Kofler hot-stage microscope and are uncorrected; boiling points are uncorrected.

Prepartion of Compounds. 2-Thiouracil was obtained from Aldrich. The following compounds were prepared using literature methods: 2,4-dichloropyrimidine (14), b.p. 99-102 °C/21 mm (lit.,[32] 100 °C/19 mm); ammonium dithiocarbamate (22), decomposed on heating without melting (lit.,[36] unstable); methylammonium N-methyl dithiocarbonate (23), m.p. 100-102 °C (lit., g); S-(beta-carboxyvinyl)-dithiocarbamic acid (24), m.p. 169-171 °C (decomp.) (lit.,[35] no m.p. reported); S-(beta-carboxyvinyl)-N-methyl dithiocarbamic acid (25), m.p. 148-150 °C (lit.,[35] no m.p. reported); 2-mercapto-4-oxo-4H-1,3-thiazine(26), m.p. 182-183 °C (lit.,[35] 184 °C); 2-mercapto-3-methyl-4-oxo-4H-1,3-thiazine (27), m.p. 72-74 °C (lit.,[35] 78 °C); 1-methyl-2-thiouracil (28), m.p. 232-233 °C (lit.,[35] 226-227 °C); 3-methyl-2-thiouracil (29), m.p. 242-244 °C (lit.,[35] 207 °C; lit.,[46] 292-294°C).

2-Methylthio-4-pyrimidone (6).- To 2-thiouracil (4) (12.8 g, 0.1 mol) and NaOH (7.6 g, 0.19 mol) in 200 ml of H_2O-EtOH (1 : 1) was added methyl iodide (11.5 ml. 0.18 mol). The mixture was stirred at 50 - 60 °C for 20 min and stored overnight at 10 - 15 °C. The precipitated solid was filtered off and washed with H_2O. The filtrate was acidified with HOAc and concentrated to approximately 50 ml. The resulting solid was isolated by filtration, washed with H_2O, and dried. The combined crops of solid were recrystallised from EtOH to give pyrimidone (6) (5.38 g, 38%) as needles; m.p. 199-201 °C (lit.,[25] 198 °C); Δ_H ($CDCl_3$/DMSO-d_6) 2.53 (3H, s, $SC\underline{H}_3$), 6.14 (1H, d, J = 6.6 Hz), 7.96 (1H, d, J = 6.6 Hz).

2-Methylthio-3-methyl-4-pyrimidone (11).- To a solution of 5N NaOH (44 ml, 220 mmol) containing 2-thiouracil (4) (10.0 g, 78.0 mmol) at 0 °C was added dropwise dimethyl sulfate (20 ml, 220 mmol). After complete addition, the mixture was heated to 70 °C, cooled to 5 °C for 2 h, and then kept at -5 °C overnight. The resulting solid was isolated by filtration, washed with cold H_2O (1 x 10 ml), and dried to give pure (11) (3.50 g, 29%) as white plates. A further portion of (11) (0.303 g, 2%) was isolated from the filtrate by saturation with NaCl, extraction with $CHCl_3$ (30 x 50 ml), rotoevaporation of extracts (1-4), and column chromatography [silica gel, $CHCl_3$-EtOAc (3:1)] of the residue; m.p. 123-124 °C (lit.,[26] 122-123 °C; $nu_{max.}$ ($CHBr_3$) 1675, 1499, 1415, 1339, 1142, 1100 cm^{-1}; $delta_H$ ($CDCl_3$) 2.55 (3H, s, $SC\underline{H}_3$), 3.49 (3H, s, $NC\underline{H}_3$), 6.12 (1H, d, J = 6.2 Hz), 7.67 (1H, d, J = 6.2 Hz).

Compound (11) was also isolated during the previous reaction procedure by evaporation of the aqueous layer to dryness after separation of the second crop of (6). After the residue was extracted with hot toluene, the solvent was evaporated yielding (11) (1.243 g, 8%).

1-Methyl-2-methylthio-4-pyrimidone (9).- This compound was isolated from the above reaction after evaporation of the $CHCl_3$ from collected extracts (5-30); recrystallisation from toluene-EtOH gave (9) (1.429 g, 12%) as white needles. An additional portion of (9) (1.385 g, 11%) was obtained from column chromatography of the residue; m.p. 168-169 °C (lit.,[26] 166-167 °C); Δ_H ($CDCl_3$) 2.58 (3H, s, $SC\underline{H}_3$), 3.55 (3H, s, $NC\underline{H}_3$), 6.00 (1H, d, J = 7.6 Hz), 7.22 (1H, d, J = 7.6 Hz).

1,2-Dihydro-1,3-dimethylpyrimidine-2-thione-4-one (10).- This product was also isolated from the above reaction. Chromatography [$CHCl_3$-EtOAc (3:1)] of the residue gave crude (10) (0.327 g, 3%) which on recrystallisation from EtOH gave an analytically pure sample; m.p. 118-120 °C (lit.,[47] 108-109 °C); Δ_H ($CDCl_3$) 3.40 (3H, s), 3.45 (3H, s), 5.83 (1H, d, J = 8.2 Hz), 7.27 (1H, d, J = 8.2 Hz).

4-Chloro-2-methylthiopyrimidine (12).- A mixture of (6) (2.00 g, 14.1 mmol) and $POCl_3$ (10 ml, 107 mmol) was heated to reflux for 1.75 h and left overnight at room temperature. The excess $POCl_3$ was evaporated and the residue shaken with ice. The mixture was made basic with 20% NaOH solution and extracted with benzene. The organic layer was separated, washed with water, dried over anh. $MgSO_4$, and rotoevaporated to give pyrimidine (12) as an oily product of purity greater than 90% as shown by ^1H n.m.r.; Δ_H ($CDCl_3$) 2.59 (3H, s, $SC\underline{H}_3$), 7.09 (1H, d, J = 5.6 Hz), 8.53 (1H, d, J = 5.6 Hz).

4-Methoxy-2-methylthiopyrimidine (13).- To a stirred solution of 4-chloro-2-methylthiopyrimidine (12) (2.24 g, 14 mmol) in 10 ml of MeOH under N_2 was added, dropwise over 15 min, 10 ml of a sodium methoxide solution [0.345 g, 15.0 mmol (Na metal)]. The solution was stirred at room temperature for 30 min and then the solvent removed by rotoevaporation. The oily mixture was extracted with $CHCl_3$. The organic layer was washed with 25% NaOH solution,

then with H_2O, and dried over anh. $MgSO_4$. Rotoevaporation of the solvent gave a residue which contained (13) (88%) and an N-alkylated derivative (12%) by ^1H n.m.r. An analytically pure sample of (13) was obtained by column chromatography [Silica gel, benzene-$CHCl_3$ (2:1)] of the crude mixture. Recrystallisation from n-pentane gave pure pyrimidine (13) as white needles; m.p. 32-33 °C (Found C, 46.1; H, 5.4; N, 18.1. $C_6H_8N_2OS$ requires C, 46.1; H, 5.2; N, 17.9%); Δ_H ($CDCl_3$) 2.58 (3H, s, $SC\underline{H}_3$), 4.02 (3H, s, $NC\underline{H}_3$), 6.46 (1H, d, J = 5.8 Hz, 5-H), 8.36 (1H, d, J = 5.8 Hz, 6-H).

2-Chloro-4-methoxypyrimidine (16).- To a stirred ice-cold solution of dichloropyrimidine (14) (7.5 g, 50.0 mmol) in 25 ml of MeOH under N_2 was added, dropwise over 2 h, 25 ml of a sodium methoxide solution [1.15 g, 50.0 mmol (Na metal)]. The mixture was stirred at room temperature overnight. The NaCl precipitate was filtered off and the filtrate concentrated on an oil bath at 130 °C. The residue was then distilled; the fraction boiling at 96-97 °C/18 mm (6.578 g) partially solidified. Trituration with n-pentane gave (16) (6.523 g, 90%) as a white solid; Δ_H ($CDCl_3$) 4.08 (3H, s, $OC\underline{H}_3$), 6.77 (1H, d, J = 6.0 Hz, 5-H), 8.11 (1H, d, J = 6.0 Hz, 6-H).

4-Chloro-2-methoxypyrimidine.- This product was also produced in the previous reaction. It was found in the filtrate from the n-pentane washings of (16). ^1H N.m.r. of the residue (0.955 g, 6%) after evaporation of the solvent from the washings showed it to be a mixture of 4-chloro-2-methoxypyrimidine (57%) and (16) (43%); Δ_H ($CDCl_3$) 4.08 (3H, s, $OC\underline{H}_3$), 7.09 (1H, d, J = 5.4 Hz, 5-H), 8.53 (1H, d, J = 5.4 Hz, 6-H).

2,4-Dithiouracil (21).- To an ice-cold solution of chloropyrimidine (16) (4.65 g, 32.0 mmol) in 20 ml of MeOH which had been saturated with H_2S was added 20 ml of sodium methoxide solution [0.74 g, 32.2 mmol (Na metal)]. The solution was then sealed in a tube and heated at 98 °C for 45 min. The vial

was carefully opened and the solvent rotoevaporated to give a residue which after purification by column chromatography [Silica gel, $CHCl_3$-MeOH 12:1)] gave starting material (16) and 2,4-dithiouracil (21) (0.598 g, 13%) as yellow needles on recrystallisation from EtOH; m.p. 264-266 °C (decomp.) [(lit.,[48] 235 °C (decomp.)].

2-Chloro-4-neopentylpyrimidine (15).- To neopentyl alcohol (50.0 g, 567 mmol) was added solid sodium methoxide prepared by dissolving Na metal (1.38 g, 60 mmol) in 100 ml of MeOH and evaporation of the solvent. The resulting solution was concentrated to approximately 30 ml by rotoevaporation and to this was added dropwise over 30 min a solution of dichloropyrimidine (14) (7.5 g, 50 mmol) in 10 ml of toluene. The solution was stirred at room temperature overnight. ^1H N.m.r. analysis on the crude product showed it not to contain any starting material. The solution was used without any further purification.

4-Neopentyl-2-thiouracil (17).- To the crude solution of chloropyrimidine (15) (as prepared above) in 50 ml of neopentyl alcohol was added N,N′-dimethylthiourea (5.21 g, 50 mmol). After being stirred at 60 °C for 15 h, ^1H n.m.r. of the mixture showed the absence of starting material. The excess alcohol was then rotoevaporated and the residue chromatographed [Silica gel, $CHCl_3$, then $CHCl_3$-isopropanol (20:1), followed by MeOH] yielding crude (17) from the MeOH fraction. Recrystallisation from MeOH gave pyrimidine (17) (0.100 g, 1%) as white needles; m.p. 198-200 °C (Found C, 54.2; H, 7.2; N, 14.0. $C_9H_{14}N_2OS$ requires C, 54.5; H, 7.1; N, 14.1%); Δ_H ($CDCl_3$) 1.00 (9H, s, $C\underline{H}_3$), 4.22 (2H, s, $OC\underline{H}_2$), 6.32 (1H, d, J = 7.2 Hz, 5-H), 7.68 (1H, d, J = 7.2 Hz, 6-H), 13.11 (1H, bs, $N\underline{H}$); Δ_c ($CDCl_3$) 26.3 (CH_3), 77.6, 100.6, 144.0, 168.7, 182.1.

2-[(Methylamino)(methylimino)methylthio]-4-neopentyloxypyrimidine (**18**).- This compound was also isolated from the above reaction mixture by evaporation of the $CHCl_3$ fraction obtained by chromatography. Rotoevaporation of the solvent and recrystallisation from n-hexane gave pyrimidine (18) (0.60 g, 7%) as white crystals; m.p. 102-105 °C Found C, 45.3; H, 5.7; N, 26.3. $C_8H_{12}N_4OS$ required C, 45.3, H 5.7; N, 26.4 %; ^1H n.m.r. ($CDCl_3$) δ, 1.03 (0H, s, $C\underline{H}_3$), 3.27 (3H,d, J = 4.8 Hz, $NHC\underline{H}_3$), 4.06 (5H, s, $OC\underline{H}_2$, $NC\underline{H}_3$), 6.46 (1H, d, J = 6.0 Hz, 5-H), 8.29 (1H, d, J = 6.0 Hz, 6-H), 12.74 (1H, $N\underline{H}$).

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Table 1. Proton Affinity, PA, (Kcal/mol)Determined by the Bracheting Method

Cpd. No.	Compound	PA	Models			PA_{cal}
			more basic[a]		less basic[a]	
2	2-hiouracil	209.1 ± 0.4	aniline	208.6	m-chloroaniline[b]	209.5	203.5
6	2-methylthio-4-pyrimidone	220.7 ± 0.1	t-butylamine	220.5	s-butylamine	220.8	212.4
5	1-methyl-2-thiouracil	214.1 ± 0.6	2-bromopyridine	213.5	p-anisaldehyde	214.7	206.9
7	3-methyl-2-thiouracil	209.1 ± 0.3	aniline	208.6	m-chloroaniline	209.5	203.9
10	1,3-dimethyl-2-thiouracil	214.1 ± 0.6	2-bromopyridine	213.5	p-anisaldehyde	214.7	206.0
11	2-methylthio-3-methyl 4-pyrimidone	217.5 ± 0.4	n_propylamine	217.0	ethylamine	217.9	212.2
9	1-methyl-2-methylthio-4-pyrimidone	233.2 ± 0.6	tri-n-propylamine	232.3	triethylamine	234.0	225.9
13	2-methylthio-4-methoxy-pyrimidine	223.0 ± 1.2	pyrrolidine	220.8	t-butylamine	225.2	214.4
17	4-neopentyl-2-thiouracil	215.2 ± 0.3	pyridazine	214.7	2-bromopyridine	215.6	215.5[d]
1	uracil	208.0[46]					
4	dithiouracil	217.0[46]					

a: Taken from Ref. 41 except when otherwise designated.
b: From Ref. 40
c: From Ref. 48
d: for 4-methyl-2-thiourcil

Table 2 Effects of Methyl Substitution on Gas Phase Proton Affinities[a]

System	PA for R = R′ = H	Δ 1st Me for R = Me R′= H	Δ 2nd Me for R = R′ = Me	Ref
2-RS-pyridine	217.0	0.4	-	38
C_6H_5NRR'	209.5	8.6	10.0	41
HCONRR′	198.4	7.0	5.6	41
2-R_2N-pyridine	223.8	-	2.7 each	41
PhCSNHR	?	4.0	-	39a
C_6H_5OR	196.3	4.0	-	41

a) All quoted in Kcal/mol units.

Table 3. Deduction of Proton Affinities of Individual Tautomers of 2-Thiouracil From PA Measurements of Compounds which Form Cations of Type **31**

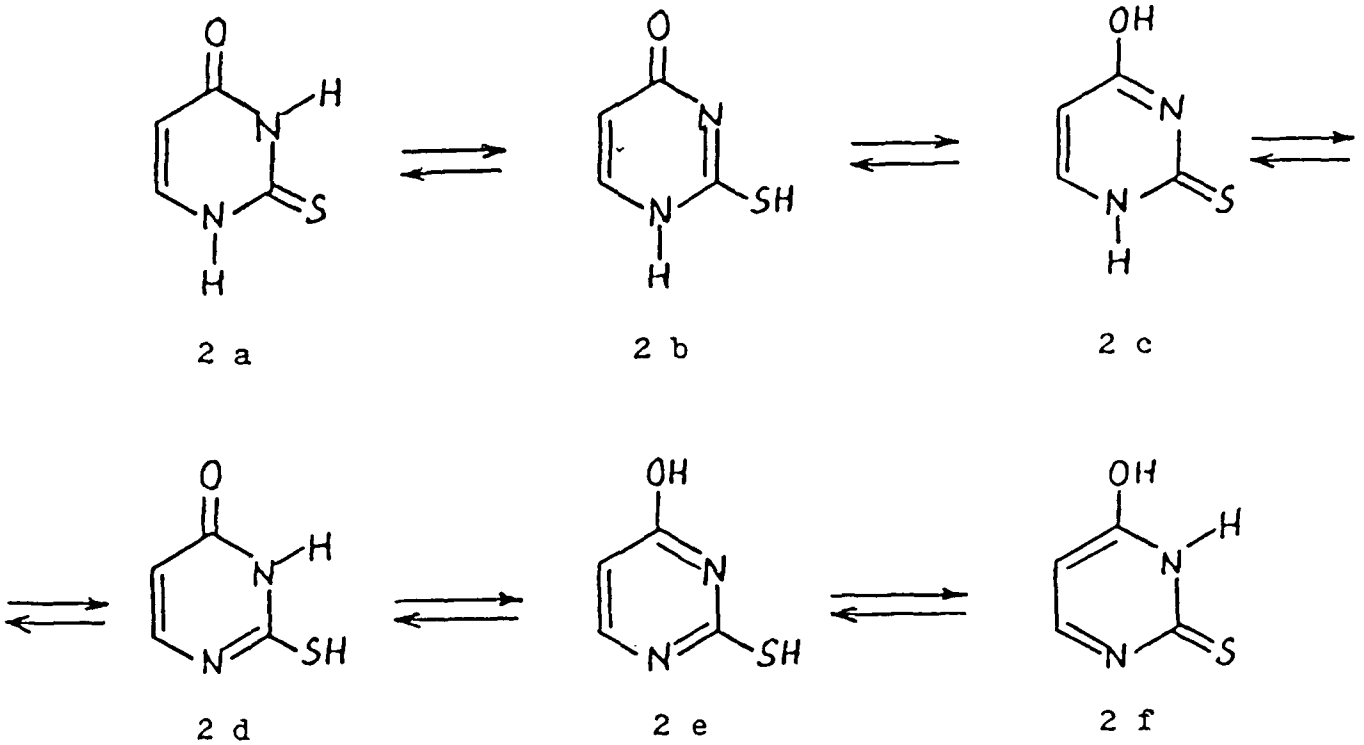
Tautomers		(Block 3 here)		
P.A. Deduced	209.1	217.6	220.3	227.8
Compound Measurement		(Block 4 here)		
PA Measured	209.1	223.0	220.7	233.2
ΔNMe	-	-	-	-5.0
ΔSMe		-0.4	-0.4	-0.4
ΔMe	-	-5.0	-	-
Relative PA	0	8.5	11.2	18.7
Relative ΔH_f [a]	0	4.9	6.2	17.0

a) From ref. 48.

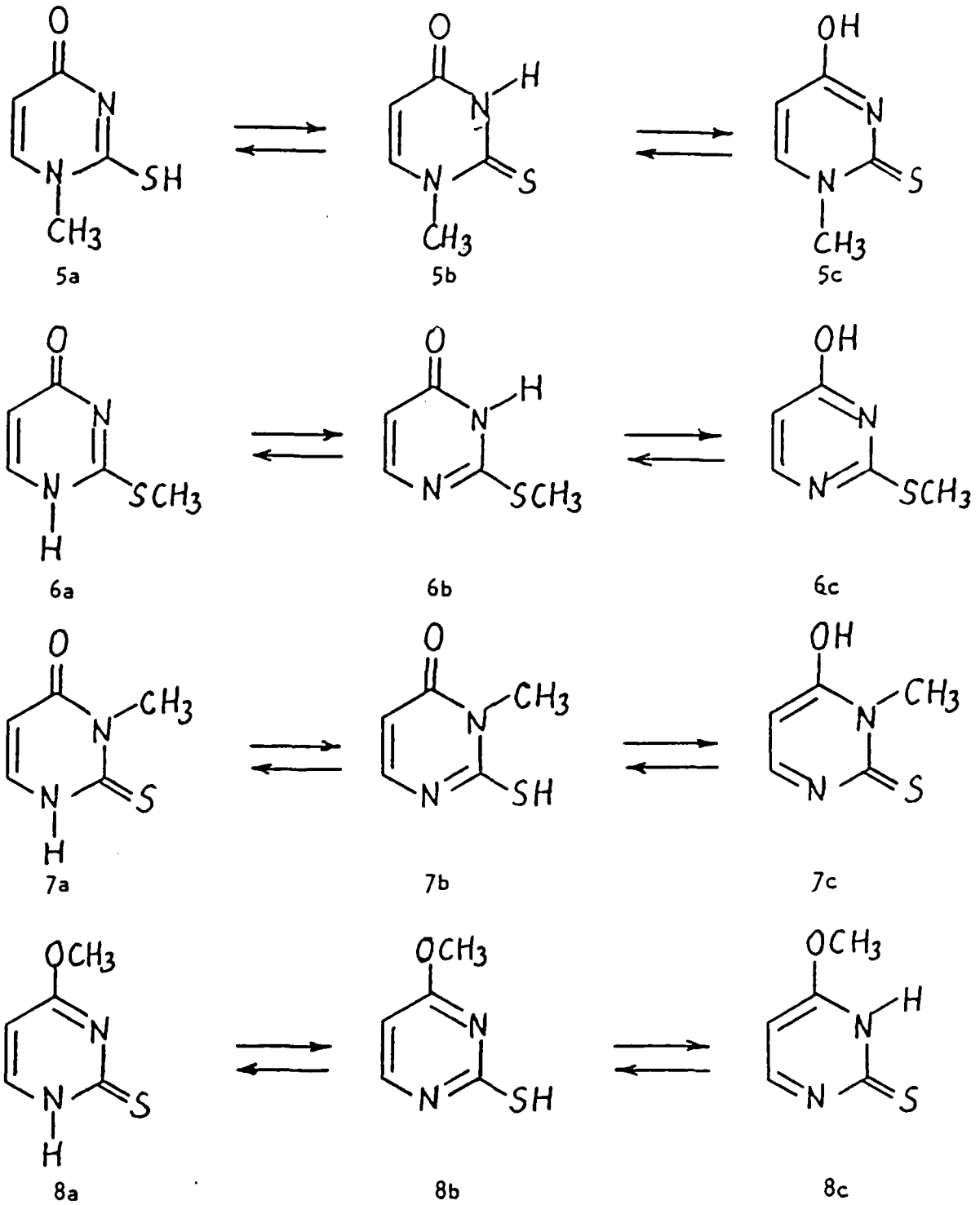
Table 4. Deduction of Proton Affinities of Individual Tautomers of 2-Thiouracil from PA Measurements of Compounds Forming Cations of Type **32** and **33**.

Tautomer	(Block 5 here)	
PA Deduced	204.1	212.1
Compound Measurement	(Block 6 here)	
PA measured	209.1	217.5
ΔNMe	-5.0	-5.0
ΔSMe		-0.4
Relative PA	0	8.0
Relative ΔH_f	0	6.2

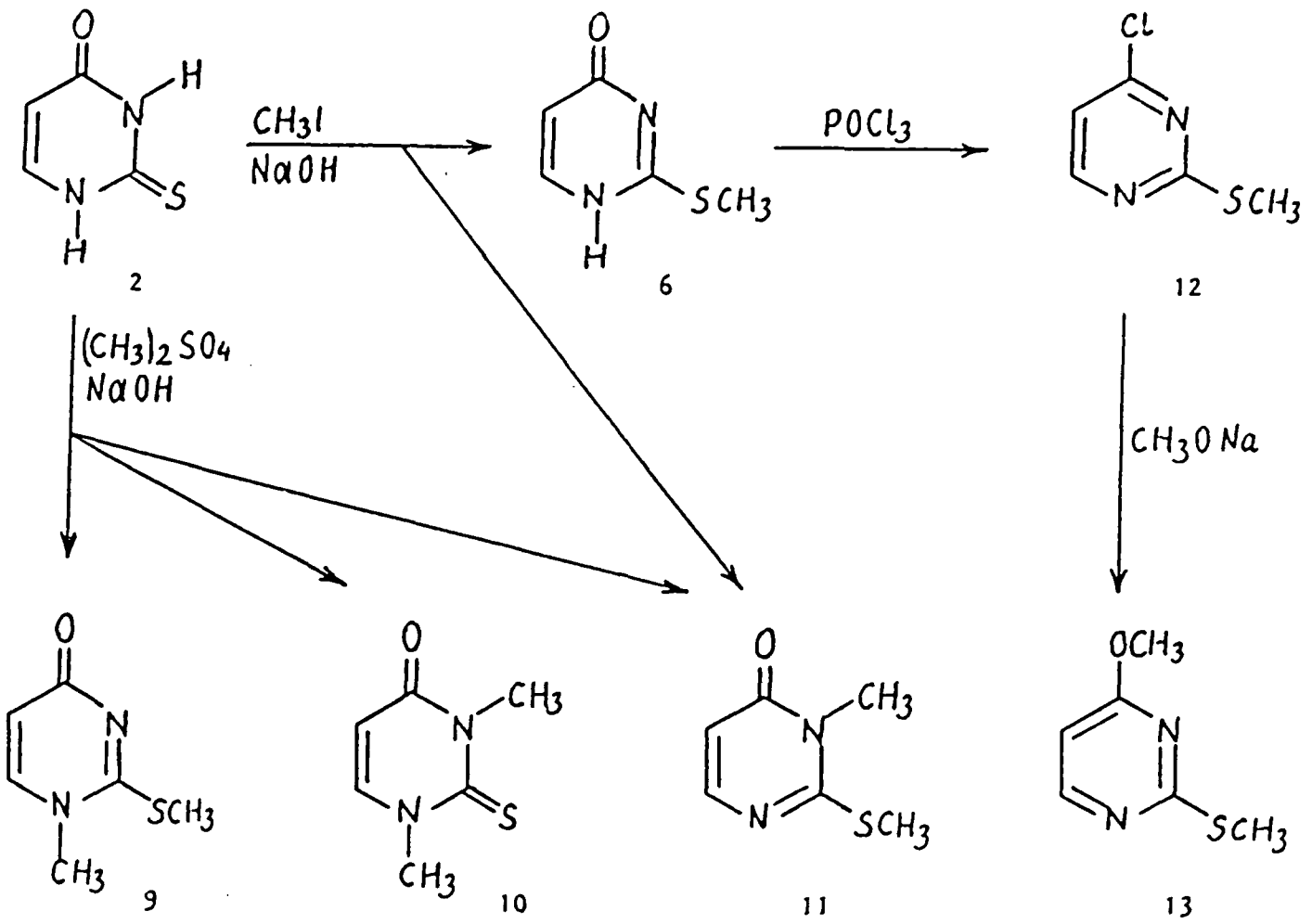
A) From ref. 4ℰ.



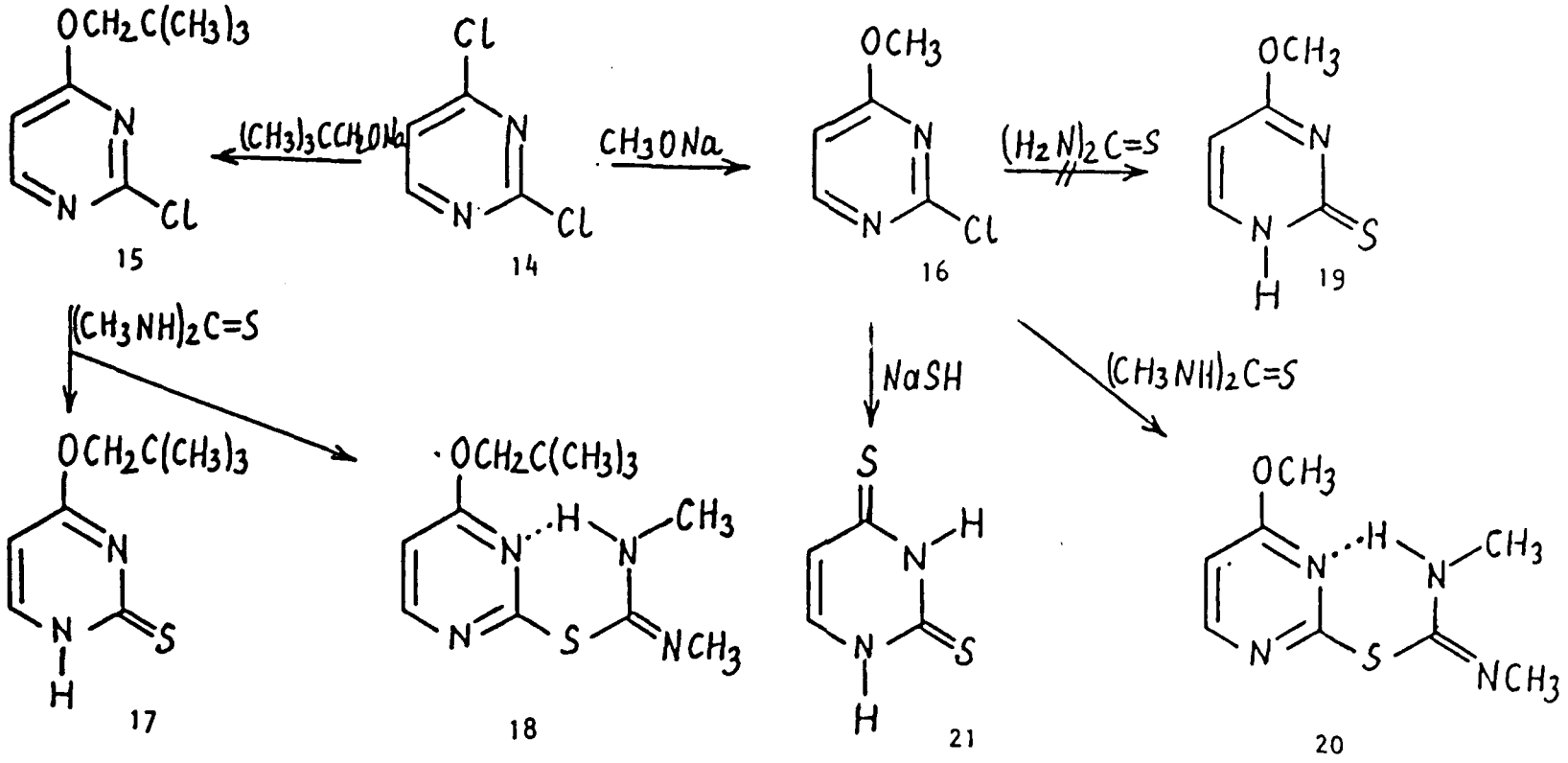
Scheme 1



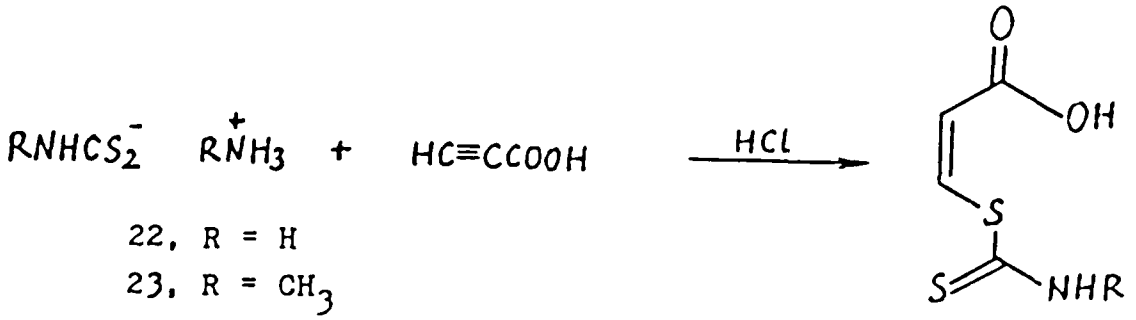
Scheme 2



Scheme 3

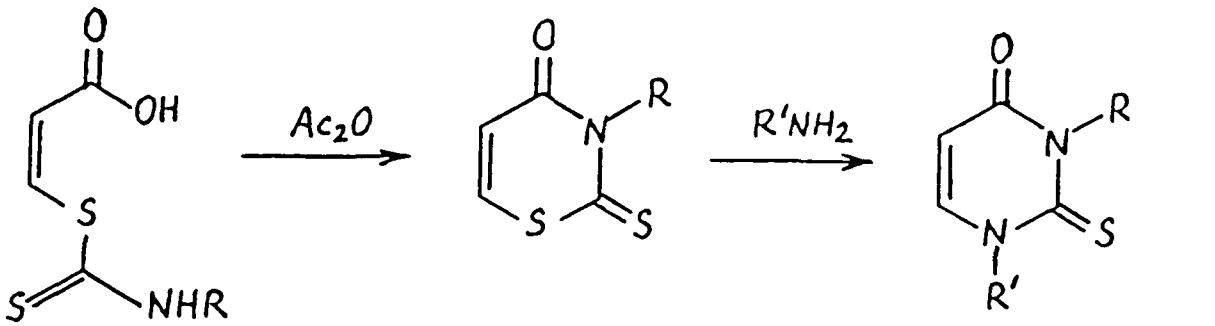

OCH2C(CH3)3
Cl
(CH3)3CCH2ONa
CH3ONa
OCH3
(H2N)2C=S
OCH3
15
14
16
19
(CH3NH)2C=S
NaSH
(CH3NH)2C=S
OCH2C(CH3)3
OCH2C(CH3)3
S
OCH3
17
18
21
20

Scheme 4



22, R = H
23, R = CH_3

24, R = H
25, R = CH_3

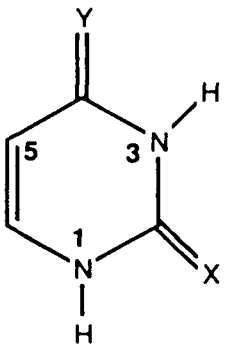


26, R = H
27, R = CH_3

28, R = H, R' = CH_3
29, R = CH_3, R' = H

Scheme 5

Block 1



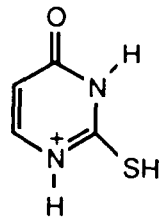
1, X = Y = O

2, X = S, Y = O

3, X = O, Y = S

4, X = Y = S

BLOCK 2



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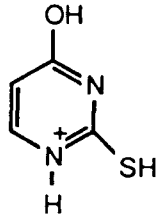
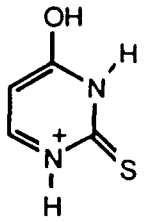
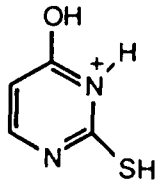


31

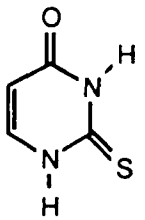


32

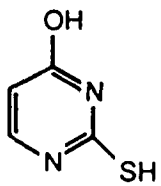


33

BLOCK 3 (to be inseted into Table 3)



2a



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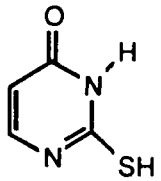
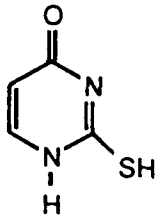
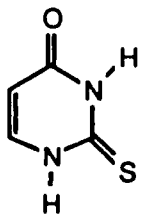


2d

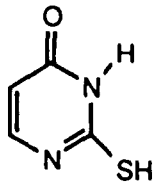


2b

BLOCK 5 (to be inseted into Table 4)



2a



2d

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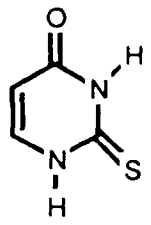
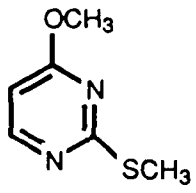


2a



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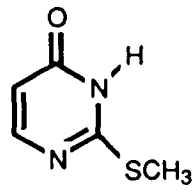
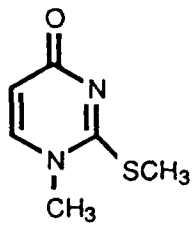
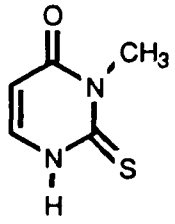


6b

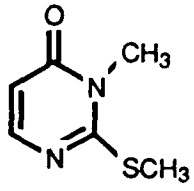


9

BLOCK 6 (to be inseted into Table 4)



7a



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